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EXAMINER

MEAH, MOHAMMAD Y

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PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

DETAILED ACTION

With supplemental amendment, filed 12/09/010, in response the final office action, mailed on 06/08/2010, the applicants cancelled claim 7 and amended claims 1-3 and 5-6. Claims 1-3, 5-6, 8-15 are currently pending in the instant application. Applicants amendment, filed 12/09/010 has been entered.

Applicants' arguments filed on 12/09/2010 have been fully considered but they are found unpersuasive. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Objection

Claim 5 is objected for reciting "within the plasmid pCS-CGndh within *Escherichia coli* DH5 α .pCS-CGndh". It is unclear if the process requires *E. coli* DH5alpha/pCS-CGndh or if it only requires the microorganism to comprise the plasmid pCS-CGndh. It is suggested to change the claim to recite "the process according to claim 1 wherein the microorganism is *E. coli* DH5alpha/pCS-CGndh" if the intended microorganism is *E. coli* DH5alpha/pCS-CGndh. If the intended microorganism is one that comprises the plasmid, the claim should be amended to recite "the process according to claim 1 wherein the microorganism comprises the plasmid pCS-CGndh". Examiner will interpret the claim as requiring the microorganism to comprise the plasmid pCS-CGndh. Appropriate correction is required.

35 U.S.C 112 1st Paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

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The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-3, 8-15 remain rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 1-3, 8-15 are directed to a method of production of amino acids by using a microorganism expressing a heterologous polynucleotide which is approximately 69.8% sequence identical to the polynucleotide of SEQ ID NO: 3 encoding a protein having NADH dehydrogenase activity.

A calculation of the T_m of the polynucleotide recited in claim 1 shows that under the hybridization conditions recited, the recited polynucleotides can be approximately 69.8% sequence identical to the polynucleotide of SEQ ID NO: 3. Using the well known equation of Meinkoth and Wahl (Current Protocols in Molecular Biology, Hybridization Analysis of DNA Blots, pages 2.10.8-2.10.11, 1993), $T_m = 81.5\text{ }^{\circ}\text{C} + 16.6 \times \log_{10}[\text{Na}^+] + 0.41 \times (\% \text{GC}) - .61 \times (\% \text{form}) - 500/L$, the corresponding T_m for the polynucleotide recited is approximately $95.2\text{ }^{\circ}\text{C}$ assuming a G+C content of 50% and neglecting the term $500/L$, where L is the length in nucleotides of the probe ($95.2\text{ }^{\circ}\text{C} = 81.5 + 16.6 \times \log_{10}[3.9 \times 2/20] + 0.41 \times (\%50) - .61(\% \text{form} = 0)$; for 2xSSC the molar concentration of Na^+ is 3.9). As known in the art, T_m is reduced by approximately $1\text{ }^{\circ}\text{C}$ for each 1% mismatching, therefore under the conditions recited (2xSSC and $65\text{ }^{\circ}\text{C}$), a

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wash at 65 °C is equivalent to approximately 30.2% mismatching ($30.2\% = 95.2^{\circ}\text{C} - 65^{\circ}\text{C}$). This level of mismatching amounts to 424 nucleotides which can be modified ($424 = 0.302 \times 1404$) within SEQ ID NO: 3 (1404 nucleotides). Thus, the genus of polynucleotides recited encompass polynucleotides having at least 69.8% sequence identity with the polynucleotide of SEQ ID NO: 3. Since each of these nucleotide mismatches can affect a codon, the genus of polynucleotides recited can encompass polynucleotides where a great majority of the mismatches affects a codon, thus these polynucleotides can encompass polynucleotides having a protein having essentially no structural similarity with the polypeptide of SEQ ID NO: 4.

As discussed in the written description guidelines the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. A representative number of species means that the species, which are adequately described, are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus. The specification teaches method of production of amino acid by using a microorganism expressing only a few heterologous DNAs encoding NADH dehydrogenases such as that of SEQ ID NO: 4. Moreover, the

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specification fails to describe any other representative species by sufficient identifying characteristics or properties to show that applicant was in possession of the claimed genus.

There is no structure-function correlation with regard to the members of the genus of polypeptides having NADH dehydrogenase activity encoded by the polynucleotides recited in the instant claims. The specification discloses the structure of a few polynucleotides encoding a polypeptide having NADH dehydrogenase activity. No disclosure of the structural features required in any polynucleotide encoding the recited proteins has been provided. Nothing is known with regard to the structural elements that are essential and those that can be modified in the polynucleotide of SEQ ID NO: 3 such that one could make a variant that encodes a protein having the desired activity. However the claimed method requires a genus of polynucleotides encoding a genus of polypeptides having essentially any structure (as for example any number of amino acid variations of SEQ ID NO: 4; encoded by polynucleotides having at least 69.8% sequence identity to the polynucleotide of SEQ ID NO: 3). This includes various allelic variants of the polypeptide of SEQ ID NO: 4 that the specification does not disclose. Therefore one of skill in the art would not recognize from the disclosure that applicants' were in possession of the claimed invention.

Applicants' are referred to the revised guidelines concerning compliance with the written description requirement of U.S.C. 112, first paragraph, published in the Official Gazette and also available at www.uspto.gov.

Claims 1-3, 5-15 remain rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of production of amino acids by using a microorganism expressing heterologous DNAs which encode the NADH dehydrogenase of SEQ ID NO: 4, does not reasonably provide enablement for a method of production of amino acids by using a microorganism expressing a heterologous polynucleotide which is approximately 69.8% sequence identical to the polynucleotide of SEQ ID NO: 3 encoding a protein having NADH dehydrogenase activity. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

Claims 1-3, 5-15 are so broad as to encompass a method of production of amino acids by using a microorganism expressing a heterologous polynucleotide which is approximately 69.8% sequence identical to the polynucleotide of SEQ ID NO: 3 encoding a protein having NADH dehydrogenase activity. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of polynucleotides of unknown structure encompassed by the claims. Since the amino acid sequence of a protein encoded by a given polynucleotide determines its structural and functional properties, predictability of which changes can be tolerated in the encoded protein's amino acid sequence to obtain the desired activity requires knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins'

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structure relates to its function. However, in this case the disclosure is limited to a few polynucleotides encoding NADH dehydrogenases, including one comprising SEQ ID NO: 3 that encodes the polypeptide of SEQ ID NO: 4. It would require undue experimentation of the skilled artisan to make and use the claimed polynucleotides encoding said polypeptides. The specification provides no guidance with regard to the making of variants and mutants or with regard to other uses. In view of the great breadth of the claims, amount of experimentation required to make the claimed polypeptides, the lack of guidance, working examples, and unpredictability of the art in predicting function from a polypeptide primary structure, the claimed invention would require undue experimentation. As such, the specification fails to teach one of ordinary skill how to make and/or use the polynucleotides encompassed by the claims.

While isolation techniques, recombinant and mutagenesis techniques are known, and it is routine in the art to screen for multiple substitutions or multiple modifications as encompassed by the instant claims, the specific amino acid positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions.

The specification does not support the broad scope of the claims because the specification does not establish: (A) regions in the polynucleotide (SEQ ID NO: 3) structure which may be modified without affecting its original activity of encoding a

NADH dehydrogenase; (B) the general tolerance of NADH dehydrogenases to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any nucleotide with an expectation of obtaining a protein having the desired biological function; and (D) sufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including polynucleotides with an enormous number of nucleotide modifications to SEQ ID NO: 3. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)).

Argument

Applicants' arguments against the rejection of claims 1-3, 5-6, 8-15 under 35 U.S.C. 112, first paragraph are not found persuasive. As explained above, the recited hybridization conditions of claim 1 result in a 30% variation of the polynucleotide molecule of SEQ ID NO: 3 anywhere within the polynucleotide. As indicated above, a polynucleotide having 30% variation within SEQ ID NO: 3 can encompass a polynucleotide which encodes essentially any protein having NADH dehydrogenase activity which has very little structural similarity with the polypeptide of SEQ ID NO: 4 or up to 140 amino acids that can be altered in any way ($140 = 30\% \times 468$).

The reference of Witkowski et al. (Biochemistry. 1999 Sep 7; 38(36): 11643-50) teaches that only a single amino acid substitution results in conversion of the activity of

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a polypeptide to a second, distinct activity (see e.g., Table 1, page 11647). Therefore one of skill in the art will need to do enormous number of experimentation to find out the coding sequence of the polynucleotide. This would clearly constitute **undue** experimentation. While enablement is not precluded by the necessity for routine screening, if a large amount of screening is required, the specification must provide a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. Such guidance has **not** been provided in the instant specification.

Claim Rejections 35 U.S.C 103a

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all

Obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 1-3, 6, 8-10 and 14-15 remain rejected under 35 U.S.C. 103(a) by Bott *et al* (*J. Biotechnol*, 2003, 129-153, from IDS) in view of Molenaar *et al* (*J. Bacteriol*, 2000, 6884-6891, from IDS), Hollander *et al* (*Appl Microbiol Biotechnol* 1994, 42, 508-515) and Nakagawa *et al* (US20020197605) for reasons of record. This rejection was discussed at length in the previous office action and discussed it again.

Bott *et al* describes the production of amino acids, such as, glutamate and L-lysine (page 130 left column, 1st pargh) by *Corynebacterium glutamicum* and that

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respiratory chain enzymes involved in the oxidative phosphorylation in the aerobic respiration of *Corynebacterium glutamicum* are useful in amino-acid production and one such enzyme is NADH dehydrogenase (abstract and FIG 1). However; Bott *et al* do not teach the method of producing amino acid by using microorganism transformed with heterologous NADH dehydrogenase derived from *Corynebacterium glutamicum*.

Molenaar *et al* teach NADH dehydrogenase gene of SEQ ID NO: 1 encoding NADH dehydrogenase (100% identical to applicants SEQ ID NO: 4) isolated from *Corynebacterium glutamicum* which is 100% identical to applicant NADH dehydrogenase gene of SEQ ID NO: 3 and Molenaar *et al* teach said NADH dehydrogenase is Type II NADH, wherein in the reaction number of proton discharged per electron is zero (page 6884, right column last paragraph). Molenaar *et al.* also teach that *Corynebacterium glutamicum* only expresses type II NADH dehydrogenase (page 6887, right column 1st paragraph).

Methods of expressing endogenous and exogenous genes in a host cell are well known in art to enhance the production of proteins and small organic compound. For example, Nakagawa *et al* teach an improved production of fine chemicals, such as, amino acid, and vitamins, by using a production strain of transformed host cell such as *E. coli* (subject matter of claims 8-9) with exogenous gene encoding desired enzymatic activities (page 7 paragraphs 0179-0191; page 14, paragraphs 0312-0313). Amino acids such as, L-lysine is industrially important chemicals.

It is well known in art that NADH is produced in several reactions in the amino acid biosynthesis pathway of *Corynebacterium glutamicum* (Hollander *et al. Appl*

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Microbiol Biotechnol 1994, 42, 508-515, Fig 1 at page 509). NADH dehydrogenase converts NADH to NAD. Hollander *et al* teach that quantitative yield of lysine can be produced from glucose in a fermentation system comprising *Corynebacterium*, if NADH and NADPH are consumed (its concentration is decreased) (last paragraph, page 514). Therefore, since type-II membrane bound NADH dehydrogenase of *Corynebacterium glutamicum* converts NADH to NAD, by doing so it depletes the NADH and increase the production of lysine from glucose. Although *E. coli* comprises two NADPH dehydrogenases (Type 1 and type II) they are different from that of NADH dehydrogenase (100% identical to applicants SEQ ID NO: 4) isolated from *Corynebacterium glutamicum* (only 27% sequence homology, page 132, Bott *et al.*). Therefore, in order to produce amino acid in large scale, one of ordinary skill in the art is **motivated** to express *E. coli* (as taught Nakagawa *et al*) with NADH dehydrogenase gene of SEQ ID NO: 1 of *Corynebacterium glutamicum* of Molenaar *et al* and use the said transformed microorganism in the method of production of amino acid.

As such it would have been obvious to one of ordinary skill in the art to use Molenaar *et al*/NADH gene of SEQ ID NO: 1 encoding a type II NADH dehydrogenase (which discharge zero proton per electron) isolated from *Corynebacterium glutamicum* which is 100% identical to applicant NADH gene of SEQ ID NO: 3 express the said gene in *E. coli* and use the transformed *E. coli* to the method of production of amino acid.

Claims 11-13 remain rejected under 35 U.S.C. 103(a) by Bott *et al* (*J. Biotechnol*, 2003, 129-153, from IDS) in view of Molenaar *et al* (*J. Bacteriol*, 2000, 6884-6891), Hollander *et al* (*Appl Microbiol. Biotechnol.* 1994, 42, 508-515) and Nakagawa *et al* (US20020197605).

The teaching of Bott *et al*, Hollander *et al* and Nakagawa *et al* is discussed above for the 35 U.S.C. 103(a) rejection of claims 1-3, 6, 8-9, 14-15. However Bott *et al*, Hollander *et al* and Nakagawa *et al* do not teach explicitly a process of producing amino acids using *Corynebacterium glutamicum* expressing heterologous NADH-II dehydrogenase gene of SEQ ID NO: 1.

Since Bott *et al* describes the production of amino acids by *Corynebacterium glutamicum* in the biosynthesis of amino acids use different respiratory chain enzymes and one of the enzymes used is NADH dehydrogenase (NADH-II), in order to further enhance the production of amino acids by *Corynebacterium*, one ordinary skill in the art is motivated to express heterologous NADH-II dehydrogenase gene of SEQ ID NO: 1 of Molenaar *et al.*) in *Corynebacterium* or *Corynebacterium glutamicum*. One of ordinary skill in the art would reasonably expect this to increase the amount of the NADH-II dehydrogenase produced in the *Corynebacterium* and therefore, enhance the amino acid production.

As such it would have been obvious to one of ordinary skill in the art to use Molenaar *et al*/NADH dehydrogenase gene of SEQ ID NO: 1 encoding type II NADH dehydrogenase isolated from *Corynebacterium glutamicum* which is 100% identical to applicant NADH gene of SEQ ID NO: 3 express the said gene in *corynebacterium* or

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Corynebacterium glutamicum and use the transformed *Corynebacterium* or *Corynebacterium glutamicum* to the method of production of amino acid, as taught by Bott *et al.*

Arguments and response

Applicants argue, at pages 7-10 of their amendment of 12/09/10, that one of ordinary skill in the art would not combine the recited prior arts in the 35 USC 103 rejection above because Nakai teaches away from using *E. coli* NADH-II in amino acid production in *E. coli*. Applicants also argue that *C. glutamicum* has 2 of the same kinds of NADH dehydrogenases found in *E. coli*. Applicants' arguments have been fully considered, but they found unpersuasive. In fact, as explained in the 103(a) rejection above, Bott *et al* and Molenaar *et al* teach that *C. glutamicum* comprises only one NADH dehydrogenase which is membrane bound NADH-II type whereas *E. coli* comprises both NADH-I and NADH-II. As argued in the previous rejection, *E. coli* comprises two NADPH dehydrogenases (Type I and type II) they are different from NADH II dehydrogenase (100% identical to applicants' SEQ ID NO: 4) isolated from *Corynebacterium glutamicum* (only 27% sequence homology to *E. coli* NADH-II, page 132, Bott *et al.*). As discussed above *Corynebacterium glutamicum* is an amino acid producing microorganism having only a type II NADH dehydrogenase. Said NADH dehydrogenase involved as a primary dehydrogenase, linked with central metabolism, in the respiratory chain of *Corynebacterium glutamicum* and its growth and for the production of amino acids. One of ordinary skill in the art would recognize that NADH-II

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dehydrogenase of *Corynebacterium glutamicum* is involved in amino acid production in *Corynebacterium glutamicum*. With regard to the teachings of Nakai *et al* (US2002/0160461), it is noted that (1) NADH-II dehydrogenase (100% identical to applicants' SEQ ID NO: 4) isolated from *Corynebacterium glutamicum* (only 27% sequence homology to that of *E. coli*) is not the same NADH-II of *E. coli*; and (2) Nakai *et al* teach that (Table 2, page 20) over-expression of cytochrome [bd-type] oxidoreductase (CYO) in *E. coli* increases the amino acid production. Production of lysine in transformed *E. coli* strain increases from .29g/L (wild-type) to .48-.53 g/L (over-expressed CYO). NADH-I is involved in cytochrome bo-type oxidoreductase pathway, not NADH-II (pages 1-2, Nakai *et al*). In Nakai *et al*'s, *E. coli* strain over-expressed with cytochrome [bd-type] oxidoreductase (CYO) uses NADH I dehydrogenase in the amino acid production, not the NADH-II. Therefore a mutation of NADH-II in the said *E. coli* strain does shows little effect on amino acid production. The CYO over-expressed *E. coli* strain produces 0.48 g/L of lysine when NADH-II of said strain is not mutated and produces 0.53 g/L of lysine when NADH-II is mutated (within experimental error 0.48 and 0.53 are same, Table 2, page 20). Applicants' argument that *Corynebacterium glutamicum* probably uses other dehydrogenases that consume NADH/NADPH in the production of amino acid is considered. However, as explained above since *Corynebacterium glutamicum* uses a type-II NADH dehydrogenase in the central metabolism, in the respiratory chain, growth and amino acid biosynthesis, one ordinary skill in the art would introduce Molenaar *et al* NADH gene of SEQ ID NO: 1 isolated from *Corynebacterium glutamicum* which is 100% identical to applicant NADH

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gene of SEQ ID NO: 3 to a microorganism to enhance the production of amino acid. Moreover as explained above NADH dehydrogenase converts NADH to NAD. Hollander *et al* teach that quantitative yield of lysine can be produced from glucose in a fermentation system comprising *Corynebacterium*, if NADH and NADPH are consumed (its concentration is decreased) (last paragraph, page 514). Therefore, since type-II membrane bound NADH dehydrogenase of *Corynebacterium glutamicum* converts NADH to NAD, by doing so it depletes the NADH and increase the production of lysine from glucose. Therefore, in order to produce amino acid in large scale, one of ordinary skill in the art is **motivated** to express *E. coli* (as taught Nakagawa *et al*) with NADH dehydrogenase gene of SEQ ID NO: 1 (encoding Type-II membrane bound NADH dehydrogenase) of *Corynebacterium glutamicum* of Molenaar *et al* and use the said transformed microorganism in the method of production of amino acid. Thus, the claimed invention remains *prima facie* obvious over the prior art of record.

Allowable Subject Matter/Conclusion

Claims 1-3, 6, 8-15 are rejected and claim 5 is objected . None of the claims are allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Mohammad Meah whose telephone number is 571-272-1261. The examiner can normally be reached on 8:30-5PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Robert Mondesi can be reached on 571-272-0956. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only.

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Mohammad Younus Meah
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